Bacterial Culture

Sterile Technique

1. Always work around a flame or in the hood
2. Flame the mouth and cap of any bottle, flask or tube upon uncapping and recapping
3. Sterilize metal instruments between uses by dipping in 100% ethanol and flaming

Bacterial Culture Maintenance

Culture cells:

1. At 36 degrees Celsius
2. Shaking at 220 rpm
3. At 10% total flask/tube volume
4. In mid-log phase($0.1 < OD600 <= 0.4$) (with OD600 = 1 ->$8.8x10^8$cell/ml)

Bacterial Culture For Gene Expression Experiments

1. Pick and individual colony from a plate and inoculate 2ml LB + amp media
2. Incubate overnight at 37 C, shaking at 220 rpm
3. Inoculate fresh media with overnight culture such that new culture has 2.5% inoculum; this is the secondary culture
4. Incubate at 37 C shaking at 220 rpm until OD600 = 0.4 (~2 hrs)
5. Inoculate 4 ml LB + amp + inducer (aTc or IPTG) with 100ul secondary culture
6. Continue cultures as described above in "bacterial culture maintenance" for 9 hrs
7. Isolate cell samples from cultures at 3, 6, and 9 hour time points
8. Remove 100ul sample aliquots from cultures
9. Pellet samples at 5K rpm for 5 minutes
10. Remove supernatant
11. Wash cells with 1 ml chilled 1xPBS, pH 7.6
12. Resuspend cells by vortexing
13. Re-pellet cells at 5K rpm for 5 minutes
14. Remove supernatant
15. Fix cells; resuspend cells in 1 ml 4% PFA (in PBS)
16. Incubate at RT for 30 minutes
17. Pellet cells at 5K rpm for 5 minutes
18. Remove supernatant
19. Resuspend cells in 1 ml 1xPBS
20. Store samples at 4 C until analysis by flow cytometry